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734 Fairmount Avenue
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Contract No. NO1-NS-5-2324
QUARTERLY PROGRESS REPORT
October 1 - December 30, 1995

Report No. 5

"SAFE AND EFFECTIVE STIMULATION OF NEURAL TISSUE"

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ABSTRACT

Work this quarter has centered on the cortical implantation of HMRI and Michigan microelectrode probes. In this series, a number of blunt (12 μ m diameter) HMRI probes did not elicit intracortical hemorrhages or neuronal damage of mechanical origin. This is in marked contrast to the hemorrhages resulting from the use of sharper (3 and 6 μ m diameter) probes. In the present series, we overlaid the operative site with fibrin glue which supplanted the reapproximation and suturing of the dura over the operative site, the degree to which this modification may have contributed to the improved outcome is uncertain. Work in these areas is ongoing.

INTRODUCTION

This is the fourth of a series of quarterly progress reports in which we have continued to investigate techniques to minimize trauma and microhemorrhages during microelectrode insertion (QPR #'s 1, 2 and 4, 1995).

In this report, we have evaluated the following factors: (1) The use of a proteolytic enzyme cocktail to soften the pial surface to permit easy entry and lessen dimpling of the surface of the cerebral cortex, (2) Evaluate the effects of tip configuration on ease of penetration and the incidence of microhemorrhages adjacent to the microelectrode tracks, and (3) Effects of elimination of suturing the dura by substituting fibrin glue as a dural replacement; the intent being to avoid movement of the microelectrodes after their insertion into the cerebral cortex.

MATERIALS AND METHODS

I. Surgical Protocol.

A craniectomy was performed over the parietal or cruciate gyrus (Table 1) and a horseshoe-shaped incision was made through the dura and the flap reflected to reveal the operative site. The probes were inserted either manually with forceps (IC 135, 136 and 137) or with the aid of an axial introducer mounted on the stereotaxic frame (SFMAI) (IC 133 and 138). In IC 137, the electrodes were implanted into the postcruciate gyrus. All other electrodes were implanted into the parietal cortex. The dura was not resutured in an attempt to obviate mechanical damage inflicted by forces transmitted to the electrodes by traction on the dura. Rather, fibrin glue was applied over the operative site, covering the tops of the electrodes.

As noted in previous series, penetration of the pia by the long, flexible photolithographic probes was difficult and resulted in bending and angulation of the probes. In an attempt to obviate this problem, a mixture of protease, collagenase and hyaluronidase was applied to the leptomeninges in IC 136 for 3 minutes on one hemisphere and 7 minutes on the contralateral side to partially digest and weaken it prior to electrode penetration. The implant duration was 24 hours for IC 133 and 138, and 4 hours for IC 135, 136 and 137. At the end of these periods, the animals were perfused transcardially through the ascending aorta using Karnovsky's fixative.

II. Electrodes

Two types of electrodes were used in this series. The HMRI electrodes were single, 2 mm long epoxylite-coated Ir shafts with diameters of 50 μm and 3, 6 or 12 μm diameter conical tips. The University of Michigan photolithographic electrodes were 4-probe linear arrays. Each silicon probe was 3.25 mm in length with rectangular cross-sectional dimensions of 15 x 150 μm .

III. Autopsy

Figs. 1-4 show the types and locations of the HMRI and University of Michigan electrodes. Four to 24 hours after its application, the fibrin glue overlying the operative site was easily reflected with no adhesion to the electrode matrices. Frequently, there were small patches of subdural hemorrhages around and under the electrode matrices. In a few instances, a punctate hemorrhage

TABLE 1A

AUTOPSY AND HISTOLOGIC FINDINGS:

HMRI ELECTRODES

TYPE ELECTRODE	IC #	HEMIS. & LOCATION	TYPE INSERTION	DURATION OF IMPLANTS (HRS)	AUTOPSY	HISTOLOGY		OTHER
						HEMORR.	NEURONS (SHRINKAGE)	
HMRI 3 µm	133	Rt. Post.	SFMAI Rapid	4	N	+	+++	—
HMRI 6 µm	133	Rt. Ant.	SFMAI Rapid	4	Hemorr. under matrix	++	+	—
HMRI 12 µm	138	L. Ant.	SFMAI Rapid	24	N	0	N	—
HMRI 12 µm	138	L. Post.	SFMAI Rapid	24	N	0	N	—
HMRI 12 µm	138	Rt. Ant.	SFMAI Rapid	24	Surface hemorr. medial to matrix	++	N	—
HMRI 12 µm	138	Rt. Post.	SFMAI Rapid	24	Surface hemorr. medial to matrix	0	N	Perivasc. cuffing (neutrophils)

N = Normal

SFMAI = Stereotaxic frame mounted axial introducer

Rapid = 108 cm/sec

+ = Slight

++ = Moderate

+++ = Marked

TABLE 1B
AUTOPSY AND HISTOLOGIC FINDINGS:
MICHIGAN ELECTRODES

TYPE ELECTRODE	IC #	HEMIS. & LOCATION	TYPE INSERTION	DURATION OF IMPLANTS (HRS)	AUTOPSY	HISTOLOGY		OTHER
						HEMORR.	NEURONS (SHRINKAGE)	
Mich. 4-probe	135	Rt. Medial	Manual	4	N	0	+ near 3 of 4 tracks	-
Mich. 4-probe	135	Rt. Lateral	Manual	4	N	0	+ near 3 of 4 tracks (1 track lost)	-
Mich. 4-probe	136	Rt. Medial	Manual	4	Leptomening. Hemorr.	Hemorr. not related to track probably due to enzymes	+ near all 4 tracks	Leptomeninges hemorrhagic. 100 x 750 μ m hemorr. b/w. 2 array
Mich. 4-probe	136	Rt. Lateral	Manual	4	N	+ 50 μ m wide adjacent to 1 track	+ near all 4 tracks	Probes bent laterally
Mich. 4-probe	137	L. Postcruc. (Medial)	Manual	4	N	0	+	Probes bent laterally
Mich. 4-probe	137	L. Postcruc. (Lateral)	Manual	4	N	0	+++ near 2 middle tracks	Probes bent laterally
Mich. 4-probe	137	Rt. Postcruc. (Medial)	Manual	4	N	0	+	Probes bent laterally
Mich. 4-probe	137	Rt. Postcruc. (Middle)	Manual	4	N	0	+	Probes bent laterally
Mich. 4-probe	137	Rt. Postcruc. (Lateral)	Manual	4	N	+ 50 μ m dia. Adjacent to track	N	Perivasc. cuffing (neutrophils)
Mich. 4-probe	138	L. Post. to HMRI electrodes	Manual	24	Hemorr. around all entry sites	0	+	Perivasc. cuffing (neutrophils)
Mich. 4-probe	138	Rt. Post. to HMRI electrodes	Manual	24	Surface hemorr. medial to matrix	0	N	Perivasc. cuffing (neutrophils)

N = Normal

surrounded the HMRI electrode entry sites (IC 138, posterior electrode site) but were not present at any Michigan probe site. The shaft of the posterior HMRI probe in IC 138 showed a coating of extravasated RBC.

IV. Tissue Processing

Blocks of control cortex and those containing the array site were resected at autopsy and processed through ascending concentrations of ethanol and embedded in paraffin.

Serial sections were taken from control blocks and those sectioned through the electrode tracks included any associated hemorrhages or other abnormalities (e.g., neuronal damage).

Alternate slides were stained with H & E and Nissl stains.

V. Results - Histological Examination.

A total of 6 HMRI single-probe implant sites were studied histologically. Eleven 4-probe photolithographic probe sites were available for histologic examination. For technical reasons, one 4-probe array site was lost to the study. The leptomeninges were examined for evidence of hemorrhage and infiltration by neutrophils or other cell types. Special attention was given to the state of preservation, the depth and direction of the electrodes and the appearance of all neurons, especially those near the track. The presence and extent of hemorrhages were compared with respect to the types of probe implanted.

A. HMRI Probe Sites.

1. 3 μ m tip diameter. IC 133 (1 implant, 4 hours duration). A narrow, 120 μ m long, hemorrhage is continuous with the upper part of the track. A few neurons along the lower end of the track are markedly flattened and hyperchromic (Fig. 5).
2. 6 μ m tip diameter. IC 133 (1 implant, 4 hours duration). At a depth of 480 μ m, the track is continuous with a defect which, presumably, is the site of a previous hemorrhage. In fact, a ruptured blood vessel communicates with the defect (Fig. 6). At a depth of 1,040 μ m, the presumptive hemorrhagic zone is present in the form of an elongated defect continuous with the track and contains some extravasated RBC. A few neurons are mechanically flattened and hyperchromic.

3. 12 μ m tip diameter. IC 138 (4 implants, 24 hours duration). Aside from one track which was continuous with a 500 μ m wide hemorrhage, all 4 tracks were accompanied by normal-appearing neurons (Figs. 7 & 8). All probe sites were accompanied by neutrophils. The leptomeninges adjacent to the electrode entry sites were infiltrated by numerous neutrophils.

B. University of Michigan Photolithographic Probe Sites (4-probe arrays).

1. 9 arrays, 4 hours duration (IC 135, 136, 137). In one experiment (IC 136), the 3-enzyme mixture applied to the pia at surgery caused marked leptomeningeal bleeding and, in some areas, extravasated blood penetrated partially or completely through the molecular layer. Near the right medial array in this same animal, a 750 μ m long by 100 to 550 μ m wide hemorrhage extended from the leptomeninges downward into the neuronal layers but was not related to any of the probes (Fig. 9). Aside from extravasated blood lying within the tracks of a few arrays, only 2 of 35 tracks showed blood in the neuropil adjacent to the tracks and in both instances the hemorrhages did not extend more than 50 μ m from the edge of the track. Vacuolations or edema of the adjacent neuropil were not present.

Neurons appeared completely normal near the probes of 2 arrays (IC 137, right lateral array, and IC 138, right hemisphere). At 6 of the 8 remaining array sites, the mildly affected neurons took the form of slightly shrunken, hyperchromic profiles lying close to the tracks (Figs. 10 & 11). These altered neurons were distributed along the entire track. At one array site, severe neuronal shrinkage and hyperchromicity were present in large numbers beside the 2 middle tracks of the lateral array in IC 137.

Bent and/or angled probes were a frequent finding and corroborated the observation at surgery of the difficulty of penetrating the pia, accompanied by flexing of the probes soon after entry (Figs. 12 & 13). The 3-enzyme mixture used in IC 136 to partially digest the leptomeninges

(in an attempt to afford easier penetration by the probes), resulted in patchy hemorrhages and a massive aggregation of neutrophils. The leptomeninges were infiltrated by numerous neutrophils and these cells were represented in large numbers on each side of the track as well as being admixed with extravasated RBC present within the track.

2. 2 arrays, 24 hours duration (IC 138). Neither array was associated with hemorrhage and only a rare neuron near one track is contracted and hyperchromic. Perivascular cuffing of blood vessels near the tracks was prominent. Overall, the tissues near all 8 tracks appeared normal.

SUMMARY

Neuronal shrinkage and hyperchromism were present at both the 3 and 6 μm diameter tipped HMRI electrode sites but not at any of the four 12 μm diameter tipped HMRI electrode sites. Of eleven 4-probe Michigan array sites, only two arrays showed completely normal-appearing neurons at all probe sites. Aside from several severely shrunken neurons near two tracks of the lateral Michigan array in IC 137, all other changed neurons showed only mildly shrunken and hyperchromic states and these were infrequently present along the entire probe sites.

The use of the enzyme mixture on the leptomeninges was not successful in appreciably lessening their resistance to penetration, especially by the flexible Michigan probes. The enzymes did succeed in partly digesting the walls of the leptomeningeal blood vessels, resulting in massive bleeding within the leptomeninges. The hemorrhages frequently extended into the molecular layer and occasionally into the neuronal layers.

A total of 5 hemorrhages were associated with the 49 implants (University of Michigan arrays and three tip configurations of the HMRI type electrode). Hemorrhages were present at three of the six HMRI probe sites, and two of these were of significant size (250 and 500 μm). One of these accompanied the 6 μm diameter tipped electrode track while the other was associated with one of the four 12 μm diameter conical tipped electrode tracks. Of the remaining three (small) hemorrhages, one was found adjoining a 3 μm diameter tipped electrode track. The remaining two were only about 50 μm in diameter and each was associated with an individual

probe site of the 43 Michigan tracks. The proportion of hemorrhages to total number of implant sites is a complete reversal of our results in three recent series of passive implants (QPR #'s 1, 2 and 4, 1995) in which hemorrhages were not avoided despite numerous variations in the parameters for implantation of the probes.

DISCUSSION

In an attempt to obviate the high incidence and severity of hemorrhages associated with penetrating electrodes, fibrin glue was used in this series to overlay the operative sites rather than reapproximating and suturing the dural flap after implantation. The latter procedure may have been responsible for movement of the indwelling probes, resulting in vascular disruption and hemorrhage. The marked reduction in the number and severity of interstitial hemorrhages (5 hemorrhages associated with 49 tracks of both the HMRI and Michigan designs) may be related to this procedural change. However, of the few hemorrhages encountered, two of them were associated with sharp (3 and 6 μm diameter) HMRI electrodes as compared to one associated with blunt (12 μm) diameter electrodes, so the use of blunter electrodes may also have contributed to the reduction in the amount of tissue injury.

Further, with respect to sharp vs. blunt HMRI electrodes, the sharper electrodes were accompanied by slight to moderate numbers of contracted, hyperchromic neurons compared to completely normal-appearing neurons at all blunt-tipped electrode tracks. It is significant that, despite their small cross-sectional dimensions, the Michigan probes were accompanied by entirely normal-appearing neurons at only 8 of 43 probe sites.

The bending/angulation of the flexible Michigan probes during implantation was corroborated by the histological observation of up to 8 cross-sectional profiles appearing in a single vertical section. This finding indicates a marked lateral angulation of all probes in both types of planar arrays. Further, on two occasions, the angled or bent probe profiles were not in line, signifying a non-uniform independent flexure of the probes.

In one animal, we noted proteolytic enzymes to digest the leptomeninges. Clearly, 7 minutes of exposure was sufficient to erode the leptomeningeal blood vessels, resulting in massive effusion of blood into the leptomeninges and even penetration downward to the neuronal layers.

Despite this, penetration by the Michigan probes remained difficult. The massive efflux of neutrophils into the leptomeninges of this animal (IC 136) reflects the marked acute inflammatory response elicited by the proteolytic enzymes. Because of the findings in this report and those reported previously (QPR #'s 1, 2 & 4, 1995), we concluded that enzyme pre-treatment of the leptomeninges before electrode penetration should be discontinued.

WORK NEXT QUARTER

The results in this series indicate the need for continued study into the use of fibrin glue in lieu of resuturing the dura over the operative site. Further work should concentrate on blunt-tipped (12 μm diameter) electrodes to explore the possible lessening of vascular damage seen with sharper (1, 3 and 6 μm diameter tipped) electrodes. Conversely, sharpening of the tips of the Michigan probes should aid significantly in their penetration of the leptomeninges but this must be weighed against the effect on the integrity of underlying blood vessels.



Fig. 1. IC 133. Autopsy view of 2 unpulsed HMRI electrodes (12 μ m diameter conical tip) in situ in the right gyrus suprasylvius. The suture attached to each matrix facilitates handling. The implant duration for this and animals IC 135 to IC 137 was 4 hours. The postimplant duration for IC 138 was 24 hours.



Fig. 2. IC 135. Autopsy view of two 4-probe Michigan arrays implanted into the right gyrus suprasylvius. The arrays are oriented in a rostral-caudal direction.



Fig. 3. IC 136. Autopsy view showing two 4-probe Michigan arrays in the right gyrus suprasylvius. The dark area around the arrays is due to hemorrhage of the leptomenigeal blood vessels caused by the lytic action of the enzyme mixture on the blood vessel walls.



Fig. 4. IC 137. Autopsy view of three 4-probe Michigan arrays implanted into the right postcruciate gyrus. In this instance, the arrays are oriented across the gyrus rather than parallel to the crown.

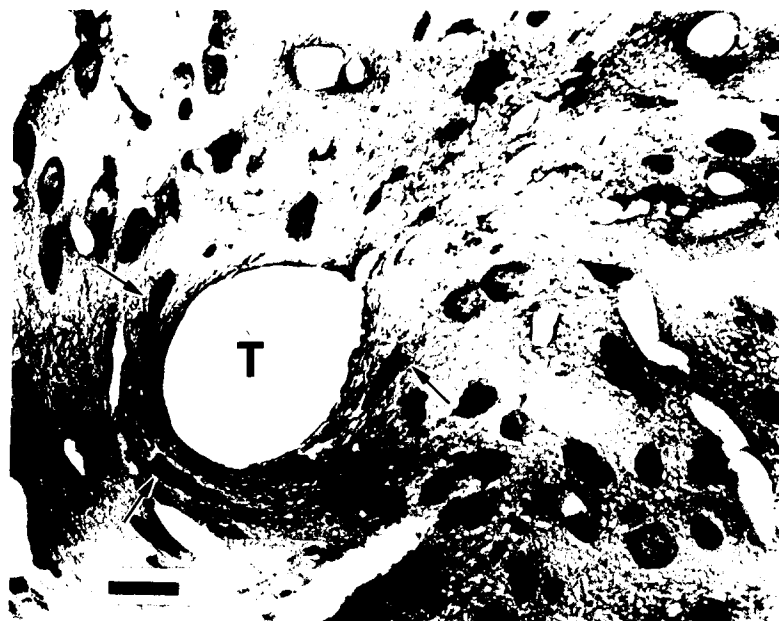


Fig. 5. IC 133. Cross-section of the track (T) of a 3 μm diameter conical tipped HMRI electrode at a depth of 600 μm . Marked neuronal flattening (arrows) is evident but hemorrhage is not present. Nissl stain. Bar = 25 μm .

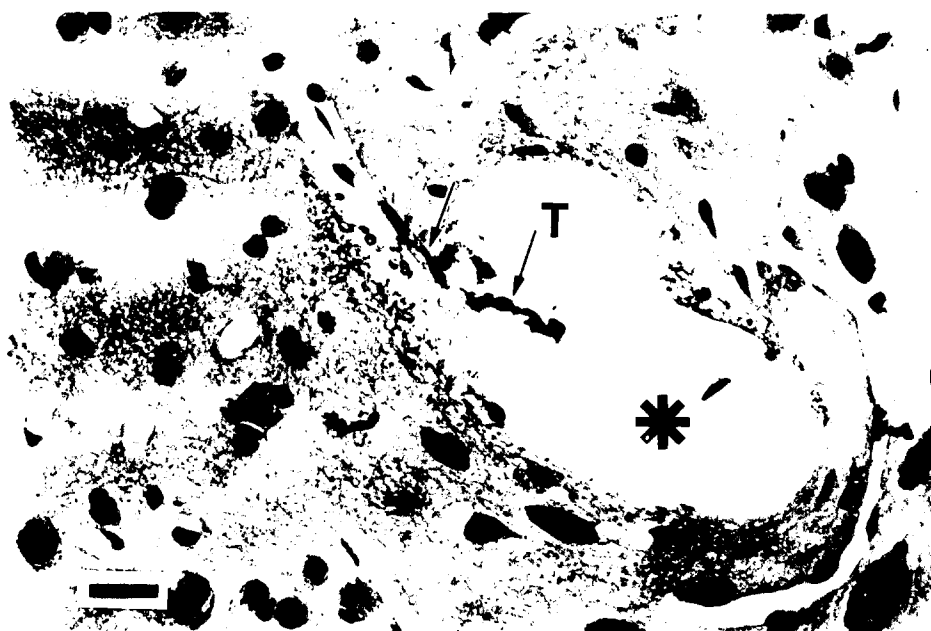


Fig. 6. IC 133. Cross-section of track of HMRI electrode with 6 μm diameter tip (T) at a depth of 480 μm . The defect (asterisk) left by a hemorrhage communicates with the track. A ruptured blood vessel (arrows) leads into the defect. A few RBC remain in the blood vessel. A few neurons are flattened and hyperchromic. H & E stain. Bar = 25 μm .

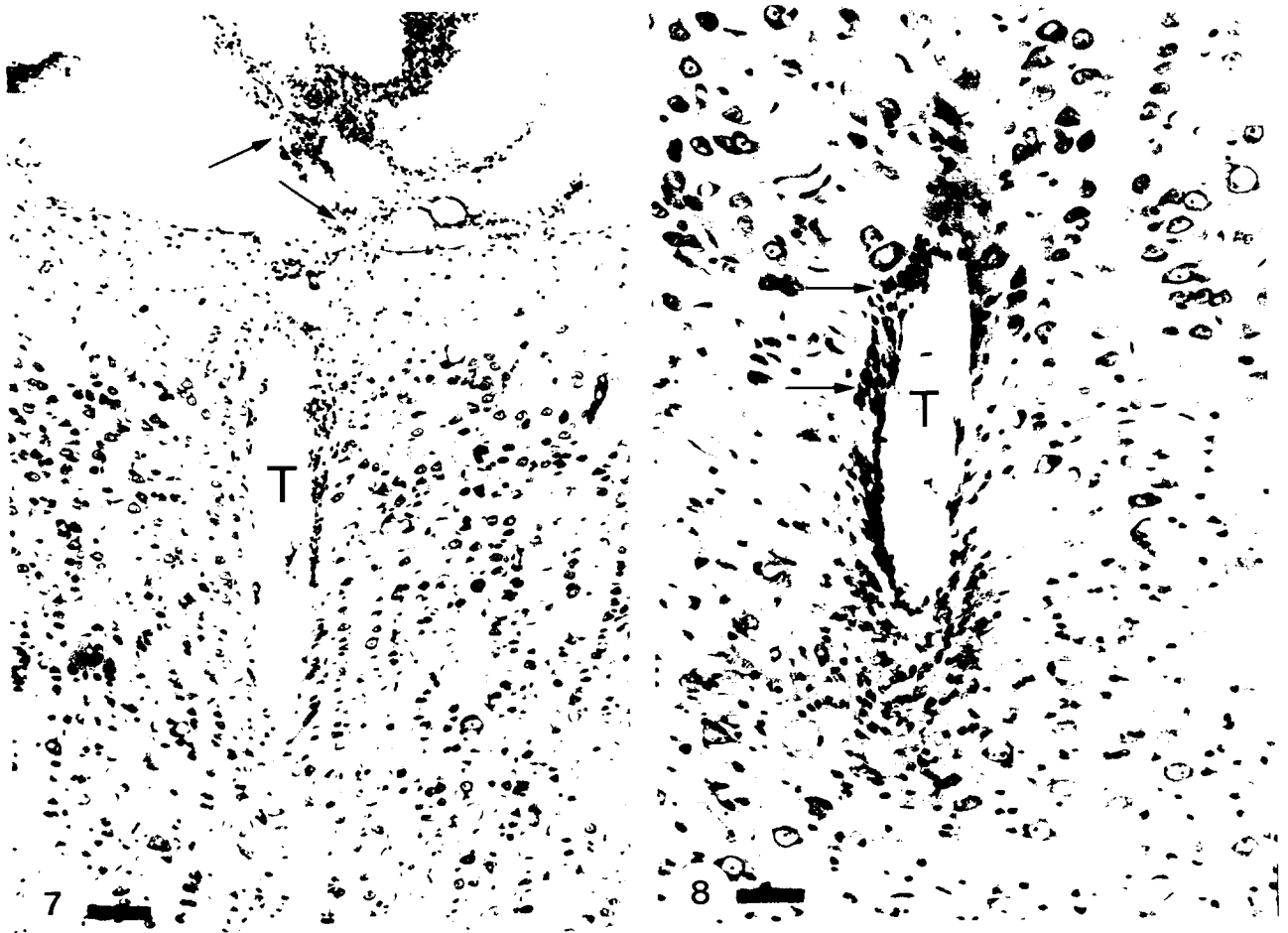


Fig. 7. IC 138. Parasagittal section through the left gyrus suprasylvius showing the upper half of the track (T) left by an HMRI probe with a 12 μm diameter conical tip. At 24 hours after implantation, numerous neutrophils have accumulated in the leptomeninges (arrows) and a few others skirt the edges of the track. All neurons appear normal and there is no hemorrhage. Nissl stain. Bar = 100 μm .

Fig. 8. IC 138. Same track (T) as that shown in the previous micrograph, including the tip at a depth of 1,550 μm . This segment is in the white matter, below most of the neurons. The neurons appear normal and hemorrhage is not present. Numerous neutrophils skirt the edges of the track (arrows). Nissl stain. Bar = 50 μm .

Fig. 9. IC 136. Tracks (T) from the medial and lateral Michigan multi probe arrays. Note the marked hemorrhage (asterisk) in the leptomeninges. At greater magnification, the leptomeninges were seen to be infiltrated by neutrophils. A 750 μm long hemorrhage (H) has dissected its way from the surface into the neuronal layers. This hemorrhage is unrelated to either array. H & E stain. Bar = 100 μm .

Fig. 10. IC 135. Midportion of one track (T) from the lateral Michigan multi probe array. This segment of the obliquely sectioned track reaches a depth of 1,800 μm . A few neurons are flattened (small arrow) while others are shrunken and hyperchromic (large arrow). Hemorrhage is not present. Nissl stain. Bar = 50 μm .



Fig. 11. IC 135. Coronal section showing one track (T) from each Michigan array portrayed in the previous photo. The longer (medial) track is 2,750 μm deep while the lateral track (right side of micrograph) is 2,280 μm deep. The medial track reaches far below the neuronal layers into the white matter while the lateral track is sufficiently far from the crown of the gyrus so that the track skirts the neurons adjacent to the sulcus. There was no hemorrhage or inflammation. H & E stain. Bar = 250 μm .

Fig. 12. IC 137. Parasagittal section passing through one track (T) of the lateral array and 3 tracks (arrows) of the adjacent (middle) array. The presence of tracks from two arrays in the same section supports the observation at surgery of the marked flexing of some photolithographic probes during penetration of the leptomeninges. In this instance, the long, vertical track reflects little, if any, flexing as opposed to the 3 profiles of severely angled probes from the adjacent array. Nissl stain. Bar = 400 μm .

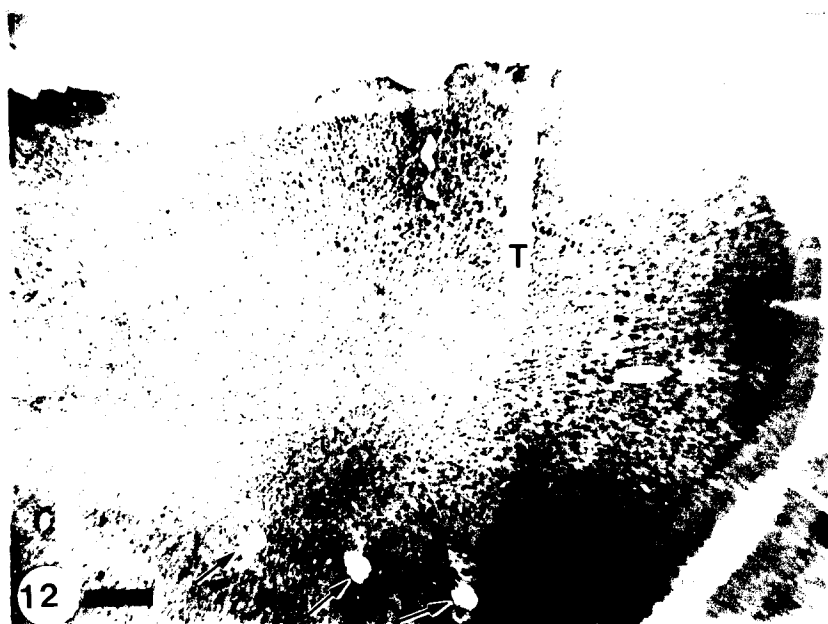




Fig. 13. IC 137. Parasagittal section through the postcruciate gyrus showing all 4 tracks of the medial array. The cross-sectional rather than vertical profiles of the tracks corroborate the observation at surgery of the marked bending of the probes during penetration of the leptomeninges. Note that the posterior track (arrow) is not in line with the other 3 tracks, indicating an increased flexion of this probe compared to the others. H & E stain. Bar = 250 μ m.